# Automated Single-Kernel Sorting to Select for Quality Traits in Wheat Breeding Lines

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**ABSTRACT**

Cereal Chem. 86(5):527–533

An automated single kernel near-infrared system was used to select kernels to enhance the end-use quality of hard red wheat breeder samples. Twenty breeding populations and advanced lines were sorted for hardness index, protein content, and kernel color. To determine whether the phenotypic sorting was based upon genetic or environmental differences, the progeny of the unsorted control and sorted samples were planted at two locations two years later to determine whether differences in the sorted samples were transmitted to the progeny (e.g., based on genetic differences). The average hardness index of the harvested wheat samples for segregating populations improved significantly by seven hardness units. For the advanced lines, hardness index was not affected by sorting, indicating little genetic variation within these lines. When sorting by protein content, a significant increase from 12.1 to 12.6% was observed at one location. Purity of the red samples was improved from ≈78% (unsorted control) to ≈92% (sorted samples), while the purity of the white samples improved from 22% (control) to ≈62% (sorted samples). Similar positive results were found for sorting red and blue kernel samples. Sorting for kernel hardness, color, and protein content is effective and based upon genetic variation.

To meet market demand, wheat breeding programs continually strive for improvements such as higher yields, increased disease resistance, better end-use quality (Baenziger et al 2001), and kernel color (Corpuz et al 1983; Keppenne and Baenziger 1990). Plants may be selected based on the presence of genes that are presumed to result in beneficial characteristics (genetic selection), or based on the expression of desirable traits (phenotypic selection). Seeds that are likely to propagate these desirable traits can be selected using molecular techniques such as marker-assisted selection (MAS) (reviewed by Baenziger et al 2006; Baenziger and DePauw 2009) or by measuring the seed chemical composition or morphological characteristics. However, many of the kernel assays developed and used to select for improved quality are either tedious, time-consuming, applicable only to large samples, or destructive. A rapid and nondestructive method to select single kernels with specific traits may help wheat breeders enrich segregating populations and reduce heterogeneity in heterogeneous advanced lines to increase the frequency of desired traits.

In this study, we investigated optical sorting techniques based on visible and near-infrared sensors, which we have previously shown to be useful for rapidly and nondestructively measuring grain characteristics. Visible and near-infrared spectroscopy (NIRS) has been used to measure single kernel traits such as color (Wang et al 1999), protein content (Delwiche and Hruschka 2000), amylose starch (Delwiche et al 2006) and hardness (Maghirang and Dowell 2003). An automated NIRS system has been used to sort wheat (*Triticum aestivum* L.) by protein content and hardness (Dowell et al 2006), and millet (*Panicum miliaceum* L.) and wheat based on the presence or absence of amylose starch (Dowell et al 2006, 2009). However, none of the previous studies have determined whether sorting based on NIRS selects for genetically controlled characteristics (e.g., genetically controlled characteristics that are passed on to progeny) as opposed to environmental characteristics. For example, sorting based on kernel protein content may reflect genetic differences in the grain or it may reflect environmental differences, such as the position of the grain in the spike or where the plant was grown in the field.

Therefore, the objective of the present research was to determine whether optical sorting based on NIRS can be used to select hard red winter wheat kernels based on genetic differences that are expressed in the measurement of kernel hardness (harder being desirable), kernel protein content (higher being desirable), and kernel color (white, red, and blue). To do this, $F_2$ bulk populations segregating for the traits of interest and advanced lines initially selected in the $F_3$ generation with minimal within-line selection thereafter were sorted for kernel hardness, kernel protein content, and kernel color. The progeny were grown to determine whether the unsorted (control) and sorted samples differed for the selected characteristics. An example of breeding methods used to create an advanced line that was released as a cultivar is the development of NE01643 (Baenziger et al 2008). If unsorted (control) and sorted samples differed, the sorting was based upon genetic (and not environmental) differences.

## MATERIALS AND METHODS

### Wheat Samples

Twenty hard red winter (HRW) wheat samples were obtained from crop year (CY) 2004, harvested in Yuma, AZ (Table I). The samples originated from 13 $F_2$ populations, with each population having one parent of soft or unknown hardness, and three advanced lines that were sorted based on hardness levels or kernel color (red, white, and blue). Samples from four additional segregating populations that had the high grain protein parent Glupro were sorted into four groups based on kernel protein content. Samples (500–1,000 g) were sorted to obtain 40 g in 2005, planted that same year in an augmented design aimed at increasing seed yield, and then harvested in 2006. In 2006, progeny of the control and sorted samples were planted in a randomized, complete block design with three replicates using standard agronomic practices for eastern Nebraska (Baenziger et al 2001) in two locations: Lincoln, where practices are most similar to southeastern wheat production in NE; and Mead, where practices are more similar to northeastern and north-central wheat production in NE. The crops were then harvested in 2007. Each plot consisted of four rows 2.4 m long with 30 cm between rows. Using a small plot combine, all plot rows were harvested and measured for grain yield. Locations were chosen because they represent different environments in eastern Nebraska.

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doi:10.1094/CCHEM-86-5-0527

To determine whether the sorting was on genetic differences among the seed, we compared the hardness index, protein content, and color class of the unsorted and sorted samples before planting to their progeny. The sample size used in the quality analysis was 1–1.5 kg. We reasoned that if the sorting was effective, the unsorted and sorted samples should differ phenotypically. If these phenotypic differences were based on genetics, then the progeny of the sorted (selected) should have more of the selected type (class) than the unsorted control.

The alternative hypothesis was that if the sorting was based on environmental (nongenetic) effects, the progeny of the sorted samples should be very similar to the progeny of the unsorted control.

## Sorting Procedures

An automated single-kernel NIR sorting system was used in all tests. Dowell et al (2006) provide a detailed description of the instrument. The system delivers single kernels into a viewing area where a NIR spectrum is collected and then sorts the kernels into one of four bins based on user-defined criteria. All calibration models for NIR sorting were developed using partial least squares (PLS) regression and Grams software from Thermo Scientific (Waltham, MA).

*Sorting by hardness.* The grain hardness calibration was developed using the 10 National Institute of Standards and Technology wheat hardness reference samples, as well as 23 additional soft and hard wheat samples obtained from the USDA Soft Wheat Quality Laboratory, Wooster, OH, and the Federal Grain Inspection Service, Kansas City, MO. For each calibration sample, 100 spectra of single kernels were collected and then averaged. For the partial least squares (PLS) analysis, the average spectrum was assigned the average hardness index obtained using the Single Kernel Characterization System (SKCS 4100, Perten Instruments, Stockholm, Sweden).

Cross-validation results for five factors resulted in an $R^2 = 0.85$ and standard error of cross-validation (SECV) of 10.41. A detailed discussion on model development for grain hardness index sorting is presented by Maghirang and Dowell (2003). The sorting criteria were adjusted to deliver an approximately equal number of kernels into each of the four bins. Bin 1 contained the softest 25% of all kernels, Bin 4 contained the hardest 25% of all kernels, and Bins 2 and 3 had intermediate levels of hardness. Because the sorting criteria were set to give an equal distribution in all bins, the sorting criteria were adjusted as needed for each sample.

*Sorting by protein content.* The protein calibration was developed from 97 hard red winter (HRW) wheat samples. Further description of these samples can be found in Maghirang et al (2006). For each calibration sample, spectral data of 100 kernels were collected and then averaged. For the PLS analysis, each averaged spectrum was assigned the corresponding bulk protein content of the sample. Cross-validation results showed that with five factors, $R^2 = 0.92$ and SECV = 0.47%. As with the sorting by hardness, the criteria for sorting by protein content were adjusted to deliver an approximately equal number of kernels into each of four bins. Bin 1 contained kernels with the lowest protein content and Bin 4 contained kernels with the highest protein content. Bins 2 and 3 contained intermediate levels of protein content. Because sorting criteria were set to give an equal distribution in all bins, they were adjusted as needed for each sample sorted.

*Sorting by color.* Two color sort calibrations were developed: 1) white versus red, and 2) blue versus red. For the white versus red calibration, spectral data were collected for 100 white kernels and 100 red kernels of Population 4441; the kernels were selected based on visual inspection. Validation results using NaOH tests showed 94% correct classification for white and 74% correct classification for red kernels. For blue versus red calibration, spectral data were collected for 100 red kernels and 100 blue kernels of Population 4507; the kernels were selected based on visual inspection. Validation tests showed a correct classification rate of 84% for red wheat and 68% for blue wheat.

For all color sorting tests, calibration models were developed by assigning a value of "1" to white kernels or blue kernels and a value of "2" to red kernels. The sorting criterion was then set so that kernels with a predicted value of ≤1.5 were considered white (for white versus red sorting) or blue (for blue versus red sorting). Kernels with a predicted value of >1.5 were considered red.

## Wheat Quality Reference Measurements

Grain texture (hardness index) was measured following Approved Method 55-31 (AACC International 2000) using the SKCS 4100 and a sample size of 300 kernels. Protein content was measured following the AACC Approved Method 39-25 using the NIRSystems 6500 (Foss North America, Silver Springs, MD) equipped with the full rectangular sample cell and two replicates.

Red or white color class was based on NaOH tests (Ram et al 2002) of 40 kernels from each of the four bins. For blue versus red sorting, we manually sorted and inspected 40 kernels from each bin.

**TABLE I**
**Identifications and Pedigrees of Populations Used to Test Near-Infrared Sorting Effects on Hardness Index, Protein Content, and Color Class**

| | Population ID | Pedigree | Sorting Criteria |
|---|---|---|---|
| Segregating populations | 4115 | NW99L7042/Madsen | Hardness |
| | 4262 | OR850513-8/NW97S182//Nuplains | Hardness |
| | 4354 | Honey/Millennium//Harry | Hardness |
| | 4356 | Honey/NI91518/Millennium | Hardness |
| | 4358 | IL95-947/Millennium//Goodstreak | Hardness |
| | 4359 | IL95-947/Millennium//Harry | Hardness |
| | 4365 | OH687/NE91518/NE00544 | Hardness |
| | 4441 | Weatherford/NuPlains//NE01643 | Hardness/Color |
| | 4444 | OR 939526)/WAHOO//NW01L2039 | Hardness |
| | 4507 | BRAVO/blue aleurone//NE99464 | Hardness/Color |
| | 4511 | MO5-1-1/ Millennium //Hondo | Hardness |
| | 4513 | MO12-2-3/*2 WAHOO | Hardness |
| | 4530 | MO124-2-2/*2 WESLEY | Hardness |
| | 4548 | GLUPRO/Empire//N87V106 | Protein Content |
| | 4549 | GLUPRO/*2 Empire | Protein Content |
| | 4550 | GLUPRO/NE99469//Goodstreak | Protein Content |
| | 4551 | GLUPRO/NE99469//Millennium | Protein Content |
| Advanced lines | Infinity | | Hardness |
| | NE01643 | | Hardness |
| | Hallam | | Hardness |

### Statistical Analyses

For the 2005 data, a *t*-test was used to determine statistical significance in all pairwise comparisons. For the 2007 data, a linear mixed model was fit to each quality parameter according to a randomized complete block design, and wheat lines were treated as random effects at both the Lincoln and Mead locations. The locations were analyzed separately due to climatic influences on wheat quality. Single degree of freedom contrasts (*t*-tests) were constructed and used to compare the mean quality parameters of sorted and unsorted treatments for the different wheat lines. The use of the 10% probability level, a less conservative test was considered because it was more important to identify differences for important traits that may be real than it is to declare real differences as being nonsignificant (economically Type I errors are unimportant, but Type II errors may reduce profitability to wheat producers) (Carmer 1976).

## RESULTS AND DISCUSSION

### Sorting by Hardness Index

The samples were sorted in 2005 to obtain the hardest 25% of all kernels for subsequent planting. For the segregating populations, the average hardness index of this hard portion selected from each sample was 75.8, which was significantly higher ($P < 0.05$) than the unsorted hardness index of 65.7 (Table II), hence NIR sorting can select for harder kernels. The hardness index range for each unsorted population was 56–79, whereas the hardness index range was 67–85 after sorting. When the unsorted and sorted portions of these 13 samples were planted, the CY 2007 harvest of those samples showed that sorting significantly ($P < 0.05$) increased the average hardness index from 69.5 in the unsorted control to 77.1 in the sorted portion at the Lincoln location, and from 54.7 in the unsorted control to 60.5 in the sorted portion at the Mead location. These findings indicate that the selection for hardness was likely based on genetic differences. The differences between locations were due to environmental effects, which are commonly observed in kernel hardness measurements (Morris et al 1999). Nevertheless, genetic improvement was evident at both locations. All of the populations showed an increase in hardness in the sorted portion when compared with the unsorted control for at least one location, except for Population 4530 (MO124-2-2 (presumed to be a soft red winter experimental line)/*2 Wesley (a hard red winter wheat cultivar) which showed a slight decrease in hardness at both locations. Because the other population (4513) involving MO124-2-2 as a parent (MO124-2-2/*2 Wahoo) exhibited an increase in hardness as a result of sorting and selection, we are unsure what made Population 4530 unique. It should be noted that population 4530 had the smallest initial improvement (2005 data) due to hardness sorting of the 13 populations that may explain the 2007 results. Overall, 12 of 13 segregating populations responded to sorting and selection with an increase in hardness.

The three advanced lines were sorted to determine whether their hardness index could be increased by selecting on possible heterogeneity within the line. In 2007, the initial sorting resulted in small and nonsignificant differences in hardness, and there was no significant difference between the sorted and unsorted selections (control) (Table II). This result was not surprising because even heterogeneous advanced lines should have a narrow range of heterogeneity compared with a segregating bulk population, especially as they were classified as being hard wheat cultivars and presumably were genetically homogenous for the hardness characteristic. The initial sorting was done on environmental (not genetically based) differences in these samples. Hence no differences were found in the following sorted progeny generations. The relative differences in these samples are an estimate of environmental variation for this trait.

The overall hardness index of the control and sorted samples harvested in CY 2007 was lower than the samples from 2005 due to environmental differences between years. This influence of the environment was not unexpected and was also reported by Morris et al (1999). However, the present results show that sorting by hardness before planting affects the hardness of the following generation when compared with control samples. Hence, selection for hardness using optical sorting of single kernels selected on genetic differences. Morris et al (1994) state that expression of the hardness gene is poorly understood, but that it is a complex

**TABLE II**
**Hardness Index (HI) of Samples Before and After Near-Infrared Sorting**

| | | 2005 | | 2007[a] | | | |
|---|---|---|---|---|---|---|---|
| | | | | Lincoln Location | | Mead Location | |
| | Population ID | Unsorted Control | High HI Fraction[b] | Unsorted Control | High HI Fraction | Unsorted Control | High HI Fraction |
| Segregating populations | 4115 | 56.0 | 67.8 | 57.7 | 65.9 | 36.7 | 47.4 |
| | 4262 | 79.0 | 85.2 | 79.0 | 79.9 | 62.0 | 66.4 |
| | 4354 | 68.3 | 74.6 | 72.8 | 75.2 | 57.8 | 57.0 |
| | 4356 | 64.6 | 74.6 | 68.3 | 75.1 | 54.6 | 62.0 |
| | 4358 | 66.3 | 76.8 | 73.3 | 83.2 | 60.1 | 63.3 |
| | 4359 | 64.0 | 77.3 | 65.0 | 72.2 | 48.3 | 57.8 |
| | 4365 | 58.9 | 67.3 | 59.7 | 77.4 | 51.2 | 57.1 |
| | 4441 | 64.4 | 77.9 | 69.1 | 75.1 | 51.1 | 54.1 |
| | 4444 | 65.0 | 76.6 | 68.4 | 78.6 | 55.0 | 61.3 |
| | 4507 | 60.4 | 76.0 | 67.4 | 77.8 | 49.3 | 62.7 |
| | 4511 | 71.9 | 82.4 | 70.6 | 83.5 | 65.5 | 68.5 |
| | 4513 | 67.8 | 77.3 | 75.3 | 84.4 | 59.8 | 68.8 |
| | 4530 | 68.1 | 71.4 | 76.3 | 73.4 | 60.2 | 59.8 |
| | Average | 65.7a[c] | 75.8b | 69.5a | 77.1b | 54.7a | 60.5b |
| | SE | | | 1.06 | | 0.94 | |
| Advanced lines | Infinity | 64.0 | 67.0 | 78.2 | 82.8 | 60.0 | 61.3 |
| | NE01643 | 59.0 | 60.0 | 80.5 | 78.2 | 66.2 | 66.3 |
| | Hallam | 60.0 | 64.0 | 65.0 | 71.0 | 52.6 | 52.6 |
| | Average | 61.0a | 63.7a | 74.6a | 77.3a | 59.6a | 60.1a |
| | SE | | | 2.31 | | 1.21 | |

[a] Unsorted and sorted kernels from 2005 were sorted and planted at two locations to yield 2007 results.
[b] Fraction comprises hardest 25% of kernels.
[c] Means within a location year followed by the same letter indicate that the mean for the high HI fraction is not significantly different at $P < 0.05$ from that of the unsorted control.

locus that codes for two related 15-kDa proteins, puroindoline a and puroindoline b. These results also agree with those reviewed and reported by Gazza et al (2008), who reported that both genetic and environmental factors determine grain hardness.

We selected the hardest kernels from genetically segregating populations due to genetic differences (heritable effects) and the environment (nonheritable effects). But while the environment did lessen the effect of selecting hard kernels for planting, we showed that sorting and selecting on hardness results in a permanent shift in the hardness index of the samples. Hardness influences breadmaking quality (Dowell et al 2008). Thus, selecting the hardest fraction from a segregating population should improve subsequent end-use quality. In a hard winter wheat breeding program, the early removal of soft kernel types means that fewer lines with unsatisfactory quality characteristics will be advanced, and this should improve the efficiency of the program by reducing the need for additional tests and resources.

## Sorting by Protein Content

The protein content of the sorted portion of the samples from 2005 increased from an average of 14.0 to 15.4% (Table III). The protein content of each of the four samples from 2005 increased by at least 1.2%, and the protein content of the sorted portion was significantly higher ($P < 0.10$) than that of the unsorted control. However, the average kernel diameter decreased significantly ($P < 0.05$) in the sorted portion of the samples from 2005 (Table IV), and we interpret the diameter to be a correlated trait resulting from inadvertent selection. One would expect that in cereal crops, higher protein kernels would have lower carbohydrate content and smaller kernel size, and therefore higher protein content (e.g., Moose et al 2004). To avoid this inadvertent selection for traits such as kernel size, the kernels could be passed through sieves to ensure similar kernel sizes before sorting. Kernels in the sorted portion also showed slightly lower weight and a slightly greater hardness index than the control portion, but these differences were not significant.

When the unsorted and the sorted high-protein kernels were planted, the 2007 harvest showed that the protein content of the sorted samples was significantly higher ($P < 0.10$) than the unsorted control at the Lincoln location (Table III), but no difference ($P < 0.10$) was seen at the Mead location. Differences in the sorted and unsorted samples were anticipated because we used lines that should have been genetically different for protein content because Glupro was one of the parents and phenotypic selection in spring wheat segregating populations has been successful (Davies et al 2006). However, protein content is strongly influenced by the environment (Peterson et al 1992; Graybosch et al 1995). Furthermore, how the environments in our study may have affected the higher protein expression in the Glupro strain is unknown. The different results between Lincoln and Mead may indicate that optical sorting for higher protein content resulted in selecting for genetic differences that were partially offset by environmentally induced differences in these populations. For example, the initial sorting may have selected higher protein kernels that achieved higher protein content by having beneficial genes from GluPro or by being the last to fill (hence smaller kernels) or both.

Ries and Everson (1973) showed that high-protein content kernels produced more vigorous seedlings and sometimes higher grain yields. Delzer et al (1995) showed that grain protein content could be increased through recurrent selection, but with a decrease in grain yield. Our results also showed no significant differences for grain yield between the unsorted and sorted high protein samples (Table III).

## Sorting by Grain Color

Populations that were segregating for kernel color were sorted to obtain enriched red and white subpopulations. Four unsorted control samples consisted of ≈22% white and ≈78% red kernels (Table V). When sorted by color, we obtained a subpopulation with 74% white kernels and a subpopulation with ≈99% red kernels; these were then planted. After sorting and planting the sub-

**TABLE III**
**Protein Content (PC, 14% mb) and Grain Yield (kg/ha) of Samples Sorted by Protein Content**

| | 2005 | | 2007 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Lincoln Location | | | | Mead Location | | | |
| | PC % | | PC % | | Yield (kg/ha) | | PC % | | Yield (kg/ha) | |
| Population ID | Unsorted Sample | Sorted Sample[a] | Unsorted Sample | Sorted Sample | Unsorted Sample | Sorted Sample | Unsorted Sample | Sorted Sample | Unsorted Sample | Sorted Sample |
| 4548 | 13.7 | 15.1 | 12.3 | 12.5 | 4193 | 4058 | 14.0 | 14.2 | 2,255 | 2,349 |
| 4549 | 13.3 | 14.6 | 11.9 | 12.4 | 3903 | 4603 | 14.3 | 14.1 | 2,430 | 2,416 |
| 4550 | 15.1 | 16.7 | 12.5 | 12.5 | 2948 | 3776 | 14.2 | 14.3 | 2,403 | 2,295 |
| 4551 | 14.0 | 15.2 | 11.7 | 13.0 | 4018 | 3977 | 14.2 | 13.9 | 2,255 | 1,716 |
| Average[b] | 14.0a | 15.4b | 12.1a | 12.6b | 3769a | 4105a | 14.2a | 14.1a | 2,335a | 2,194a |
| SE | | | 0.30 | | 4.31 | | 0.15 | | 1.69 | |

[a] Top 25% of kernels by protein content were selected from samples sorted and planted in 2005 at two locations to yield 2007 results.
[b] For protein content or grain yield within a location and year, means followed by the same letter are not significantly different at $P < 0.10$. Means are not significantly different at $P < 0.05$.

**TABLE IV**
**Average Single Kernel Weight, Diameter, and Hardness Index in Unsorted Samples and in the High-Protein Content Portion of Sorted Samples[a]**

| | Weight (mg) | | Diameter (mm) | | Hardness Index | |
|---|---|---|---|---|---|---|
| Population ID | Unsorted Sample | Sorted Sample | Unsorted Sample | Sorted Sample | Unsorted Sample | Sorted Sample |
| 4548 | 43.8 | 40.5 | 2.87 | 2.65 | 73.2 | 72.4 |
| 4549 | 41.3 | 35.7 | 2.77 | 2.55 | 72.1 | 75.2 |
| 4550 | 37.9 | 35.0 | 2.66 | 2.51 | 68.8 | 67.1 |
| 4551 | 36.7 | 32.9 | 2.61 | 2.46 | 77.7 | 78.2 |
| Average[b] | 39.9a | 36.0a | 2.73a | 2.54b | 73.0a | 73.2a |

[a] Each sample was sorted to obtain 25% of the total mass with the highest protein content. Samples from CY 2005.
[b] Means for weight, diameter, and hardness index followed by the same letter are not significantly different at $P < 0.05$.

samples, kernels from the unsorted control had 8.5–11% white kernels, corresponding to a loss of 11–14% of original white kernels; the unsorted control also had 89–91.5% red kernels. However, the portions that had been sorted to enrich the percentage of white kernels and red kernels before planting yielded ≈62% white kernels and ≈92% red kernels. The percentage of white kernels among harvested samples was ≈10% less than the proportion of white kernels among the samples sorted before planting. This result is surprising because the percentage of white kernels is expected to increase with inbreeding (in later progeny generations). Nevertheless, the trend toward fewer white kernels in the sorted sample is similar to that observed in the control sample. There are two possible explanations for these results: 1) color misclassifications (overestimations) in the initial white kernel selected populations, such that the white kernel percentage was higher than expected; or 2) white kernels were lost from both the unsorted and sorted populations for unknown reasons. The main advantage of sorting for white kernels is that two generations after sorting, the population had >60% white kernels compared with 10% in the unsorted populations, representing a six-fold enrichment for this class. For a plant breeder developing white wheat cultivars, these data mean the breeder has a six-fold better chance of selecting white seeded lines for plant breeding. The percentage of red kernels in the sorted sample from 2005 was 98.8% but this dropped to 90% in the progeny from 2007. This reduction in the percentage of red kernels was expected because the red kernel color trait is dominant and many of the originally sorted red kernels were heterozygous. Thus, some of their progeny would be white-kernel-producing plants. Interestingly, after the 2007 harvest at Mead, the red sorted and unsorted control had similar or slightly higher percentages of red kernels, indicating that sorting for red kernel types is not as effective as sorting for white kernels. It is also possible that if the samples from 2005 had been sorted more carefully, better selection for the desired types could have been achieved. Table VI shows that the grain yields (kg/ha) from the red samples were significantly greater than the yields from the white samples. This result is somewhat unexpected because a high-yielding white seeded wheat cultivar (Nuplains) was used as a parent and we find no evidence in the literature of lower yield with white-seeded cultivars. It is possible that the first parent (Weatherford, a wheat cultivar adapted to Pacific Northwest production) in the cross was not adapted to our Great Plains conditions. This result may also reflect the small number of samples we tested or possibly some environmental condition that preferentially and deleteriously affected the selected white-seeded progeny population (e.g., sprouting, though this trait was not measured because it did not seem to be present).

Similar results were observed when sorting blue from red kernels in segregating populations. The unsorted control contained ≈40% blue and ≈60% red kernels in 2005 (Table VII). The blue portion of sorted samples from 2005 averaged 83% blue kernels before planting, but contained 61–68% blue kernels in CY 2007. This result was expected because the blue aleurone trait is dominant and it masks the red kernel color genes in segregating populations and will continue cause some misclassifications. In later generations, with additional inbreeding, the proportion of red kernels should increase.

This result is in agreement with the results of Keppenne and Baenziger (1990), who found that the blue aleurone trait exhibits a gene dosage effect where the number of *Ba* genes in the endosperm determines the final kernel color and that blue kernels are often underestimated when visually scored. The genetics of this trait are complex; it is an endosperm trait which in grasses means that it exhibits triploid genetics. There are four progeny classes: deep blue (*BaBaBa*), blue (*BaBaba*), light blue (*Bababa*), and nonblue (*bababa*). The underestimation of the percentage of

**TABLE V**
**Percentage (%) of White and Red Kernels in Wheat Samples Before and After Harvesting**

| | | | | | Harvested Samples (2007) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Lincoln Location | | | | Mead Location | | | |
| | Preplanting (2005) | | | | White | | Red | | White | | Red | |
| Population ID[a] | White Control | White Sorted | Red Control | Red Sorted | Control | Sorted Before Planting | Control | Sorted Before Planting | Control | Sorted Before Planting | Control | Sorted Before Planting |
| 4441-H1 | 22 | 65 | 78 | 97 | 11 | 70 | 89 | 93 | 8 | 71 | 92 | 91 |
| 4441-H2 | 23 | 80 | 77 | 100 | 9 | 47 | 91 | 95 | 8 | 42 | 92 | 93 |
| 4441-H3 | 21 | 70 | 79 | 98 | 14 | 58 | 86 | 91 | 7 | 64 | 93 | 92 |
| 4441-H4 | 23 | 80 | 77 | 100 | 10 | 73 | 90 | 91 | 11 | 67 | 89 | 92 |
| Average[b] | 22.3a | 73.8b | 77.8a | 98.8b | 11.0a | 62.0b | 89.0a | 92.5b | 8.5a | 61.0b | 91.5a | 92.0a |
| SE | | | | | 3.05 | | 1.07 | | 3.23 | | 0.90 | |

[a] Samples are from Population 4441 after sorting into four hardness fractions.
[b] Means followed by the same letter are not significantly different at $P < 0.05$ when comparing means within a year or within a location.

**TABLE VI**
**Grain Yield (kg/ha) at Two Locations of White and Red Fractions Before and After Sorting Using a Single Kernel Near-Infrared System**

| | Lincoln Yield (kg/ha) | | | Mead Yield (kg/ha) | | |
|---|---|---|---|---|---|---|
| Population ID[a] | Unsorted | White Portion | Red Portion | Unsorted | White Portion | Red Portion |
| 4441-H1 | 3,883 | 4,092 | 4,200 | 3,264 | 2,746 | 2,820 |
| 4441-H2 | 4,092 | 3,203 | 4,300 | 2,840 | 2,463 | 2,746 |
| 4441-H3 | 4,334 | 3,843 | 4,206 | 2,968 | 2,288 | 2,934 |
| 4441-H4 | 3,897 | 4,220 | 4,213 | 2,719 | 2,497 | 2,941 |
| Average[b] | 4,051ab | 3,843a | 4,233b | 2,948a | 2,497b | 2,860ac |
| SE[c] | | 174 | 122 | | 100 | 118 |
| SE[d] | | 180 | | | 101 | |

[a] Samples from 2005 were sorted and planted to yield the samples for 2007. Four samples from Population 4441 after sorting into four hardness fractions.
[b] Means for yield within a location, means followed by the same letter are not significantly different ($P < 0.05$).
[c] Standard error of mean differences between unsorted (control) and sorted (white/red) portions within a location.
[d] Standard error of mean differences between yields of white and red portions within a location.

TABLE VII
Percentage (%) of Blue and Red Kernels in Wheat Samples Before and After Harvesting

| | Preplanting (2005) | | | | Harvested Samples (2007) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Lincoln Location | | | | Mead Location | | | |
| | | | | | Blue | | Red | | Blue | | Red | |
| Population ID[a] | Blue Control | Blue Sorted | Red Control | Red Sorted | Control | Sorted Before Planting | Control | Sorted Before Planting | Control | Sorted Before Planting | Control | Sorted Before Planting |
| 4507-H1 | 43 | 84 | 57 | 100 | 9 | 62 | 91 | 97 | 13 | 70 | 87 | 96 |
| 4507-H2 | 40 | 75 | 60 | 95 | 23 | 63 | 77 | 92 | 19 | 68 | 81 | 96 |
| 4507-H3 | 37 | 90 | 63 | 98 | 19 | 59 | 81 | 89 | 16 | 68 | 84 | 92 |
| 4507-H4 | 39 | 82 | 61 | 96 | 23 | 61 | 77 | 94 | 26 | 66 | 74 | 91 |
| Average[b] | 39.8a | 82.8b | 60.2a | 97.3b | 18.5a | 61.3b | 81.5a | 93.0b | 18.5a | 68.0b | 81.5a | 93.8b |
| SE | | | | | 2.56 | | 2.52 | | 2.24 | | 2.53 | |

[a] Four samples from Population 4507 after sorting into four hardness fractions.
[b] Means followed by the same letter are not significantly different ($P < 0.05$) for means within a year or within a location.

TABLE VIII
Grain Yield (kg/ha) at Two Locations for Blue and Red Wheat Fractions Before and After Sorting Using a Single Kernel Near-Infrared System

| | Lincoln Yield (kg/ha) | | | Mead Yield (kg/ha) | | |
|---|---|---|---|---|---|---|
| Population ID[a] | Unsorted | Blue Portion | Red Portion | Unsorted | Blue Portion | Red Portion |
| 4507-H1 | 3,937 | 3,924 | 2,921 | 2,382 | 2,181 | 2,705 |
| 4507-H2 | 4,072 | 4,233 | 3,829 | 2,719 | 2,140 | 3,022 |
| 4507-H3 | 4,267 | 3,702 | 4,018 | 2,443 | 2,140 | 2,463 |
| 4507-H4 | 3,594 | 3,903 | 4,065 | 2,369 | 2,080 | 2,295 |
| Average[b] | 3,971a | 3,944a | 3,708a | 2,477a | 2,133b | 2,625c |
| SE[c] | | 192 | 246 | | 101 | 77 |
| SE[d] | | 240 | | | 106 | |

[a] Samples from 2005 were sorted and planted to yield samples for 2007. Four samples are from Population 4507 after sorting into four hardness fractions.
[b] Means for yield within year and within location followed by the same letter are not significantly different ($P < 0.05$).
[c] Standard error of mean differences between unsorted (control) and sorted (blue/red) portions within a location.
[d] Standard error of mean differences between yields of blue and red portions within a location.

genetically blue kernels is presumably due to a misclassification of some blue kernels, most likely the visual misclassification of light-blue kernels as nonblue. The percentage of red kernels (the recessive class) in the subpopulations did not increase with additional inbreeding, which supports the hypothesis that some phenotypically red kernels were actually genetically light blue but indistinguishable from the red kernels in the optical sorting.

Considering the subpopulation sorted for red kernel color, the red portion of the 2005 samples contained ≈93% red kernels in CY 2007 (Table VII), which indicates that selection for the recessive red color was more successful. As with the red and white samples, the blue and red portions of the harvested CY 2007 samples had lower blue and red kernel frequencies when compared with kernel frequencies before planting. However, the kernel frequencies are ≈40% better for the blue samples and ≈10% better for the red samples when compared with the unsorted control.

Table VIII shows that the blue samples gave lower yields than red or unsorted samples at the Mead location, but similar yields to the red and unsorted samples at the Lincoln location. The blue aleuronic trait comes from an alien chromosome introgression (Keppenne and Baenziger 1990), which may carry deleterious genes for grain yield.

## CONCLUSIONS

The SKNIR system was effective at sorting kernels by hardness, protein content, or grain color, and it was effective at enabling selection for permanent increases in the expression of these trains in progeny. When sorting kernels by hardness, the average HI of the wheat samples harvested in 2007 for segregating populations increased by approximately seven hardness units at both of the field locations tested. For the advanced lines, for which most traits were genetically fixed, hardness index was not affected by sorting at either location, either initially or in the progeny. When sorting by protein content, difference in progeny of sorted and unsorted protein content samples was observed at one of two locations in the 2007 harvest. The selection for higher protein content might be improved by reducing inadvertent selection for smaller kernels. When sorting by color, the frequency of red, white, or blue wheat improved up to 40 percentage points in subsequent crop years.

One advantage of enriching a population for desirable traits is that a breeder can more easily select for desirable lines in the sorted populations that have those traits. The chance of identifying an improved line can be increased with sorting, which will improve the efficiency of the selection process. In this study we showed that kernel sorting for hardness, kernel color, and protein content was based upon genetic differences, and hence can benefit plant breeders who are selecting for these desirable traits.

## ACKNOWLEDGMENTS

We thank Bill Berzonsky, South Dakota State University, and Janet Lewis, Michigan State University, for comments on early versions of the manuscript, and Taylor McFall, Tom Robison, Lei Gong, and Kevin Fay for assistance in sorting, grain quality analysis, and data management, and Mark West for helping with statistical analysis.

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[Received January 30, 2009. Accepted May 4, 2009.]